PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	10/673,629	CERTIFICATE OF MAILING
Applicant	Hector F. DeLuca et al	I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 28th day of February, 2005.
Filed	September 29, 2003	
Title	Use of 2-Methylene-19-Nor-20(S)-1α,25-Dihydroxyvitamin D_3 to Increase bone Strength	
TC/A.U.	1617	
Examiner	Hui, San Ming R.	Dorothy A. Hauser February 28, 2005
		Dorothy A. Hauser Date
Docket No.	1256-00923	

DECLARATION OF HECTOR F. DELUCA

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Hector F. DeLuca, hereby declare as follows:

1) That I am one of the named inventors in the present application.

2) That I have read and I am familiar with the Patent Office Action dated November 30, 2004, and the prior art reference cited therein, namely U.S. 5,843,928, which has been applied in the Office Action against the claims of the present patent application.

3) That I conducted and supervised a series of tests in order to determine, evaluate and compare the HL-60 cell differentiation activity of the following compounds:

a) 1α,25-dihydroxyvitamin D_3;

b) 2-methylene-19-nor-1α-hydroxyhomopregnacalciferol, referred to herein as 2MP;

c) 2-methylene-19-nor-20(S)-1α-hydroxy-bishomopregnacalciferol, referred to herein as 2MbisP; and

d) 2-methylene-19-nor-1α-hydroxypregnacalciferol, referred to herein as 2Mpregna.

4. It is well known that vitamin D must be metabolized in vivo to its biologically active form, namely, 1α,25-dihydroxyvitamin D_3, before it can properly function in the human body, and thus, this vitamin D native hormone is used as the standard against which all other vitamin D analogs are typically compared.

5. The analog 2MP is covered by the generic structure in the referenced U.S. Patent 5,843,928 when R_6 and R_8 are both hydrogen, Z is Y and Y is methyl.

6. That the analog 2MbisP is covered by the generic formula in U.S. Patent 5,843,928 when R_6 and R_8 are both hydrogen, Z is Y and Y is the illustrated radical when R_1, R_2, R_3, R_4 and R_5 are all hydrogen and m and n are both 0.

7. That the analog 2Mpregna is covered by the generic formula in U.S. Patent 5,843,928 when R_6 and R_8 are both hydrogen, Z is Y and Y is hydrogen.

8. That the HL cell differentiation activity of all of these compounds were measured by nitroblue tetrazolinium reduction in accordance with the procedures set forth in Perlman et al, Biochemistry, 29, 190-196, 1990.

9. That the results of the HL cell differentiation tests are presented in Table 1 attached hereto.

10. That with respect to the compound 2MP, the data show that its HL-60 cell differentiation activity is clearly less than the native vitamin D hormone as evidenced by the fact that 2MP requires a higher concentration than 1α,25-dihydroxyvitamin D_3 ($6x10^{-9}$M versus $4x10^{-9}$M) in order to obtain the same results as 1α,25-dihydroxyvitamin D_3 (the EC_{50} value).

11. That with respect to the compound 2MbisP, the data show that its HL-60 cell differentiation activity is clearly less than the native vitamin D hormone as evidenced by the fact that 2MbisP requires a higher concentration than 1α,25-dihydroxvyvitamin D_3 ($6x10^{-9}$M versus $4x10^{-9}$M) in order to obtain the same results as 1α,25-dihydroxvyvitamin D_3 (the EC_{50} value).

12. That with respect to the compound 2Mpregna, the data show that its HL-60 cell differentiation activity is clearly less than the native vitamin D hormone as evidenced by the fact that 2 Mpregna requires a higher concentration than 1α,25-dihydroxyvitamin D_3 ($2x10^{-8}$M versus $4x10^{-9}$M) in order to obtain the same results as 1α,25-dihydroxyvitamin D_3 (the EC_{50} value).

13. That the data presented in Table 1 demonstrate that although all three of the compounds 2MP, 2MbisP and 2Mpregna have some HL-60 cell differentiation activity, and are thus not devoid of such activity, all three of these compounds are less active than 1α,25-dihydroxyvitamin D_3 in HL-60 cell differentiation activity.

14. That the HL-60 cell differentiation activity data for the presently claimed compound 2MD set forth in Figure 5 of the present patent application, show that 2MD is 10-100 times more active than 1α,25-dihydroxyvitamin D_3 in causing HL-60 cell differentiation, and thus based on these data, one skilled in the art would more likely choose 2MD over any of the above three analogs to 2MP, 2MbisP, or 2Mpregna to treat leukemia, colon cancer, breast cancer and/or prostate cancer.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Dated: _____ By: _____
Hector F. DeLuca

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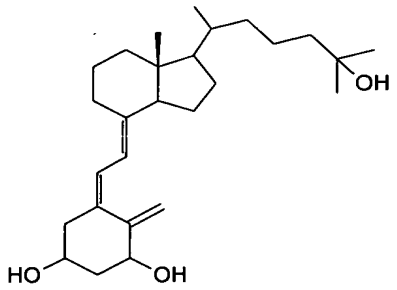
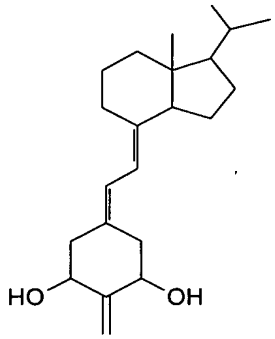
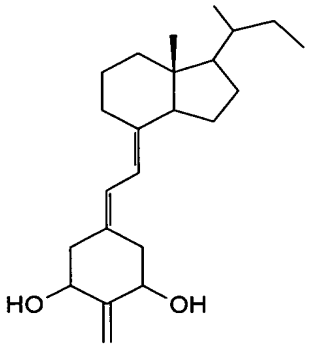
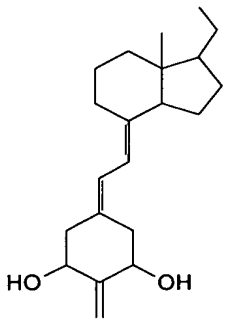
TABLE 1

SUMMARY OF HL-60 CELL DIFFERENTIATION ACTIVITY OF VARIOUS VITAMIN D ANALOGS

Compound	Chemical Structure	HL-60 Differentiation EC_{50}, M
$1\alpha,25(OH)_2D_3$		4×10^{-9}
2MP		6×10^{-9}
2MbisP		6×10^{-9}
2Mpregna		2×10^{-8}